Attorney Docket No. 29666/35415A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Box Response - No Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on this date: June 26, 2003
Williams *et al.*	
Serial No. 09/529,053	
Filed: 11/27/02	
For: Anti-Viral Uses of Leflunomide Products	
Group Art Unit: 1617	Michael F. Borun, Reg. No. 25,447 Attorney for Applicants
Examiner: S. Wang	

Declaration of W. James Waldman, Ph.D.

Box Response - No Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, W. James Waldman, Ph.D., declare that:

1. I currently hold tenured appointments as Associate Professor in the Department of Pathology and the Department of Molecular Virology, Immunology and Medical Genetics at the Ohio State University College of Medicine and Public Health where I have served as a faculty member since 1993. I also serve as Director of Pathology Graduate Studies and chair the Pathology Graduate Studies Committee. I received the Bachelor of Science (B.S.) degree in Radiologic Technology and Education from the University Without Walls of Ohio in 1978, a Master of Science (M.S.) degree in Radiation Biology in 1980 from The Ohio State University School of Allied Medical Professions, and a Doctor of Philosophy (Ph.D.) degree in Pathology in 1990 from The Ohio State University College of Medicine and Public Health Department of Pathology. I completed post-doctoral research training in virology and viral/transplantation immunology at The Ohio State University College of Medicine and Public Health, Department

of Pathology, in 1993. I have accumulated 18 years of research experience in the study of human herpes viruses, including herpes simplex virus and cytomegalovirus, focusing upon viral pathogenesis, viral immunology, viral interactions in the immunosuppressed organ transplant recipient, and anti-viral drug discovery and preclinical development.

2. I am a co-inventor of the subject matter disclosed and claimed with the above-referenced Application. I have read the Office Action of February 26, 2003 wherein claims 16, 17, 19, 20, 21, 24, and 25 were rejected under 35 U.S.C. §103(a) upon the Examiner's assertion that the claimed subject matter was rendered obvious by the disclosures of Weithmann *et al.*, U.S. Patent No. 5,556,870 (hereafter "Weithmann") in view of Flamand *et al.*, *J. Virol.*, *65*:5105-5110 (1991) (cited as CAPLUS Abstract, AN 1991:581163) and, with respect to all claims listed above except for claim 19, in view of Hammer, *AIDS*, 10:suppl. 3, s1-s11 (1996). The Examiner stated the following position:

> Weithmann *et al.* teach a method of treating disorders in which interleukin 1 beta is involved. The disorders includes viral infections, such as HIV or hepatitis, comprising administering leflunomide to the patient. *See*, particularly, the abstract and the claim. The dosage may range from 3-50 mg daily, but may be higher if required. *See*, particularly, column 3, lines 7-16.
>
> Weithmann *et al.* does not teach expressly the amount effective to inhibit viral virion assembly. However, the optimization of a result effective parameter, e.g., effective amount for a therapeutical dosage of a known therapeutical agent, is considered within the skill of the artisan. *See*, *In re Boesch and Slaney*, (CCPA) 204 USPQ 215. Further, treating a disease with an agent in a host would lead the agent contacting the pathogenic cell. A method known to be useful for treating viral infection would have been reasonably expected to be useful for prophylactic purpose. Further, known antiviral agents would have been reasonably expected to be effective *in vitro* against virus. Finally, since leflunomide is effective against virus through different mechanism, it would have been reasonably expected to effective against those virus with resistance to antiviral agent that inhibit viral DNA replication.

3. I have reviewed U.S. Patent 5,556,870 ("Weithmann") and, on the basis of my experience and training, respectfully disagree with the Examiner's position.

4. Weithmann actually contains no disclosure or suggestion that any leflunomide product possesses anti-viral activity. Further, the *in vitro* experimental results set out in Weithmann do not constitute any credible scientific basis whatsoever for the proposed therapeutic utility (i.e., reducing elevated interleukin 1 beta levels) in animals, including humans. Weithmann

acknowledges that leflunomide (HWA 486) is rapidly metabolized upon administration to form an active metabolite (A771726) (column 1, lines 9-37). That fact is supported by articles published at that time [such as Lucien *et al.*, *Therapeutic Drug Monitoring*, 17:454-459 (1995) which states that leflunomide conversion to its metabolite commences immediately and that by 7 hours the significant kinetic factor is A771726 degradation, as opposed to the conversion of leflunomide to its metabolite (*See*, p. 458)]. Notwithstanding that fact, Weithmann maintains that leflunomide, but not its metabolite, has activity in inhibiting cytokine "synthesis and liberation," stating:

> Thus, it was found, in accordance with the invention, that leflunomide exerts a strong inhibitory effect on the synthesis and liberation of cytokines from blood cells, whereas the leflunomide metabolite does not exhibit this advantageous effect. (Column 1, lines 45-49; Emphasis supplied.)

Weithmann then "demonstrates" an IL-1β reduction effect in an *in vitro* procedure involving a specially-prepared isolated blood cell fraction which has a reduced capacity to metabolize leflunomide. (*See* Example 1 and column 3, lines 23-26.)

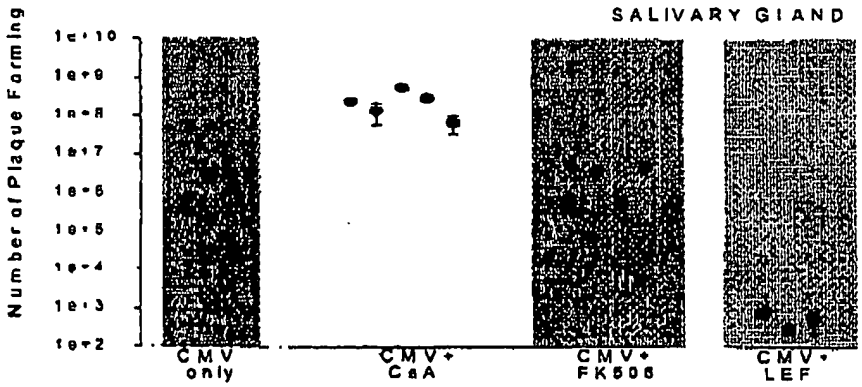
5. Thus, Weithmann's proposal for the use of leflunomide to treat a variety of diseases (including viral diseases) generically characterized by the presence of IL-1β production in "humans and animals" never addresses how to simulate his special *in vitro* test conditions *in vivo*, i.e. how to prevent leflunomide from being metabolized and "inactivated" promptly upon administration in humans and animals. Most significantly, Weithmann never proposes that leflunomide has any direct action or effect on virus replication. Contrary to the position taken by the Examiner, treatment with leflunomide is not established by Weithmann as "a method known to be useful for treating viral infection."

6. In my experience, any potential *in vivo* effect of leflunomide and its metabolite on IL-1β production (which effect is certainly *not* proven by Weithmann's experiments) would be as an outcome of the known immunosuppressive/anti-inflammatory activity of those products. As of the date of my invention, it would not have been expected that an anti-inflammatory/ immunosuppressive agent would also be an anti-viral agent. Indeed, it is well documented in the literature that immunosuppressive drugs enhance prospects for development of viral disease (*See*, Tab A for example references).

anti-CMV agent, but no significant inhibition by FK 506 or cyclosporine A, known immunosuppressive drugs.

8. We have also investigated whether there is any correlation between anti-inflammatory agents and anti-viral properties exists *in vivo*. The results of this work are in press (Waldman, *et al.*, Am. J. Transplant). Briefly, we heterotopically transplanted Lewis rats with Brown Norway (BN) hearts, inoculated them with a rat CMV (RCMV, Maastricht strain, 1 x 10^6 plaque-forming units/animal, i.p.) and treated them by gavage with 20 or 30 mg/kg/day of leflunomide or vehicle alone (5 animals/treatment group) beginning the first day of transplantation (in press, Waldman *et al.*, Am. J. Transplant). Control groups were likewise transplanted and inoculated with RCMV but received either cyclosporine A (CsA) at 20 mg/kg/day for 21 days, tacrolimus (FK506) at 1 mg/kg/day for 21 days, or no immunosuppression. While non-immunosuppressed animals acutely rejected the BN hearts by day 7, all hearts were beating strongly in rats treated with leflunomide, CsA, or FK506 through day 21 when the animals were euthanized. Immunohistochemical straining of tissue sections prepared from salivary gland, lung, liver, and spleen of CsA-and FK506-treated animals demonstrated widespread RCMV antigen positivity. In contrast, RCMV-positive cells were rarely observed in tissues recovered from leflunomide-treated rats. Likewise, plaque assay of tissue homogenates revealed up to a 6 log reduction in virus yield from tissues recovered from leflunomide-treated animals compared to those treated with CsA or FK506, Figure 2 below.

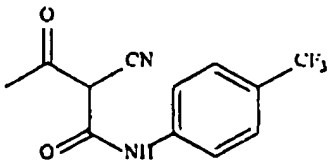
FIGURE 2



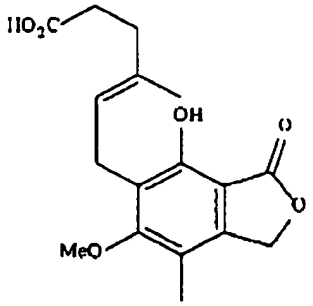
These studies again directly demonstrate the unique bifunctional utility of leflunomide as both an immunosuppressive and anti-viral agent.

9. I am aware of no data in the literature (whether *in vivo* or *in vitro*) or in practice clinically that correlates immunosuppressive effect to useful anti-viral activity, with the exception of *in vitro* reports on mycophenolic acid or mycophenolate mofetil (MMF) (Roche Pharmaceuticals) inducing apoptosis of activated CD4+ T lymphocytes (major host cell for HIV) and inhibiting HIV isolation from CD4+ T cell populations. (Chapuis *et al.* Nat Med 2000; 6: 762-768, (2000) *see* attached Tab D.) MMF by itself exerts no detectable anti-viral activity. While this agent has been demonstrated to *potentiate* the anti-herpesvirus activity of acyclovir, ganciclovir, penciclovir, and lobucavir *in vitro* and in a murine model of HSV-1-induced cutaneous lesions (Neyts *et al.*, Antimicrob Agents Chemother., 42: 216-222 (1998)); there is no evidence for such potentiation in clinical studies. Indeed the incidence and severity of herpesvirus disease in patient populations treated with MMF and anti-herpesvirus drugs has been greater than or equal to that of patients treated with other immunosuppressive drugs (*See* Tab B for exemplary references). Further, MMF is structurally distinct from leflunomide as can be seen from the side-by-side comparison below, Figure 3.

FIGURE 3



MMF

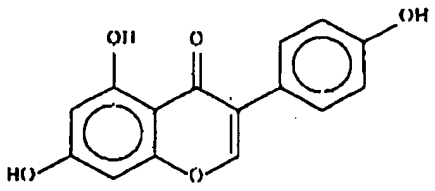


Leflunomide

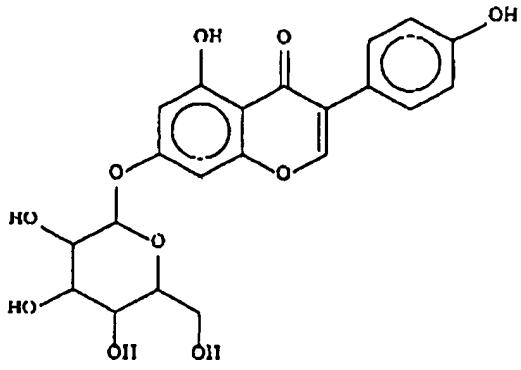
10. As stated in our Application (page 1, lines 13-21), leflunomide products are known to be protein tyrosine kinase inhibitors. I have become aware during the prosecution of this Application of the existence of publications addressing anti-viral effects of other structurally distinct protein tyrosine kinase inhibitors with mechanisms of action that inhibit the viral DNA

or RNA synthesis. Typical publications are noted in Tab C. Inhibition of polynucleotide synthesis is not the mechanism of action of the leflunomide products claimed in our Application, and it is because of leflunomide's different mode of action that utility is indicated for treatment of viral strains that are resistant to known polynucleotide synthesis inhibitors. I have also become aware of two studies presenting data correlating protein tyrosine kinase inhibitors and anti-viral properties [Yura *et al.*, Arch. Virol., 132: 451-461 (1993) and Yura *et al.*, Intervirology, 40(1): 7-14 (1997)] wherein a mechanism of action other than by inhibition of polynucleotide synthesis is proposed. I believe both are distinct from our invention. The first Yura *et al.* publication referenced above tests four structurally-related isoflavinoids, specifically genistein, prunetin, genistin and daidzein (*See* structures in Figure 4 below). Two of these structures, genistein and prunetin, showed anti-viral activity, while the related two structures did not and also did not have protein tyrosine kinase inhibiting activity. Due to the amount of difficulty in predicting whether such highly structurally similar compounds will have anti-viral efficacy, I would not expect this paper to suggest that other, non-structurally related protein tyrosine kinase inhibitors, such as leflunomide products, might have anti-viral properties.

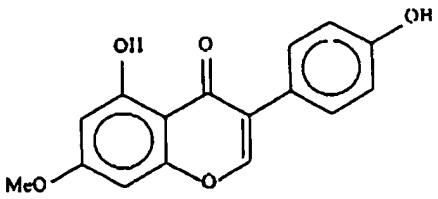
FIGURE 4:



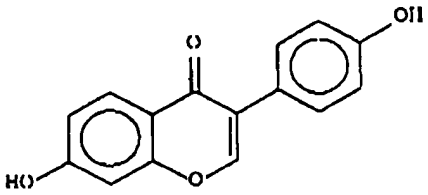
Genistein, (5,7,4'-trihydroxyisoflavone), anti-viral effect



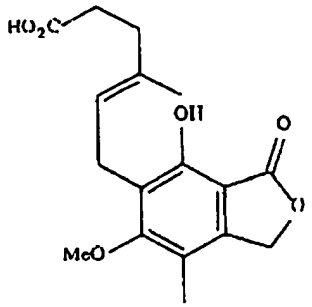
Genistin, (glucosyl-7-genistein), no anti-viral effect



Prunetin, (5,4'-dihydroxy-7-methoxyisoflavone), anti-viral effect



Diadzein, (7,4'-dihydroxyisoflavone), no anti-viral effect



Leflunomide

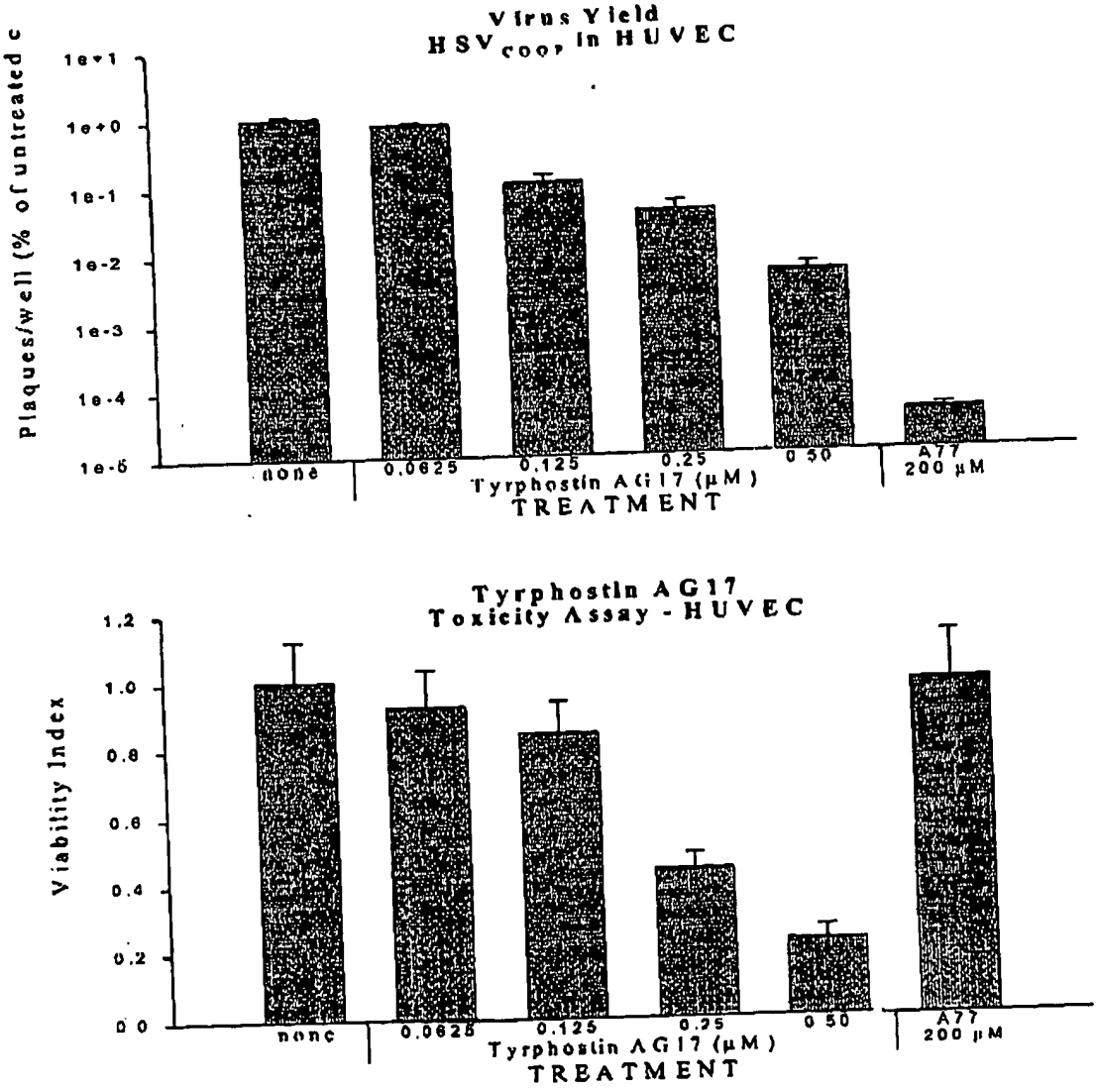
11. As for the second Yura *et al.* publication, I recently tested the tyrophostins, AG17 (EMD Biosciences, CALBIOCHEM, San Diego, CA) described therein along with the metabolite of leflunomide, A77 1726 (Aventis, NJ) for antiviral activity at non-toxic doses. The Material Safety Data Sheet from the manufacturer said AG17 is highly toxic. Therefore, I first determined what a non-toxic dosage of AG17 was by growing human umbilical vein endothelial cells (HUVEC) to confluence in 96-well microtiter plates, followed by 48 hours of incubation in culture medium supplemented with various concentrations of tyrophostin AG17 ranging from 0.0625 μM to 2.0 μM (4 replicate wells/concentration). For comparison additional microcultures

were incubated with 200 μM A77 1726 (Aventis, NJ). Cells incubated in medium alone served as negative controls. As positive controls for toxicity, cells were incubated in medium supplemented with 0.3% H_2O_2 for 6 hours prior to assay. Toxicity was measured by a colorimetric assay based upon the conversion of a light-yellow non-toxic tetrazolium compound (MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)2H-tetrazolium) into a darker colored formazan product. This conversion is catalyzed by dehydrogenase enzymes resident in living, metabolically active cells. The quantity of formazan product, as measured by photoabsorbance at λ 490 nm, is directly proportional to the number of living cells in microculture wells (Cory *et al.*, Cancer Commun 3: 207-212 (1991)). Following 48 hours of incubation under the conditions described above, culture medium was removed from microwells and replaced with phenol red-free medium (100 μL/well, 20 μL of MTS/PMS (Promega, Madison, WI) was added to each well and plates were incubated for 30-60 minutes at 37°C. Photoabsorbances (λ 490 nm) of individual microculture wells were measured with an MRX microplate reader (Dynex, Chantilly, VA), and means and standard deviations were calculated for each set of 4 replicate wells. To correct for background absorbance by dead cells, the mean absorbance values of the H_2O_2-treated wells (100% toxicity) were subtracted from values determined for the other treatment groups and the untreated controls. Values were then normalized to that of the untreated cells. The data of this toxicity study is graphically presented in the bottom portion of Figure 5. HUVEC viability is reduced to ~40% by exposure to 0.25 μM AG17, and to ~10% in the presence of 1.0 μM. Even at the dose of 0.0625 μM slight toxicity was detected. The metabolite of leflunomide ("A77" in the graph) showed no detectable toxicity at a concentration of 200 μM.

The antiviral activity of tyrophostin AG17 was then measured against herpes simplex virus (HSV) *in vitro*, in virus yield assays, by growing HUVEC cells to confluence in 24-well culture plates, then pre-treating such plates for 30 minutes with various concentrations of tyrophostin AG17 (0.0625-1.0 μM) or culture medium alone. For comparison additional microcultures were incubated with 200 μM A77 1726 (Aventis, NJ). Following pre-treatment, monolayers were rinsed twice with phosphate buffered saline (PBS), inoculated with clinical isolate HSV_{COOP} (0.05 PFU/cell), and incubated for 1 hour at 37°C. Inocula were then removed and monolayers were rinsed twice with PBS before supplying culture wells with medium supplemented as described above. Cultures were incubated for 48 hours at 37°C, then harvested

and assayed for plaque formation (Knight *et al.*, Transplantation, 71: 170-174 (2001)). The results are set out graphically in the top portion of Figure 5. Data points represent mean plaque numbers from triplicate wells ± 1 SD, normalized to values calculated from untreated, infected controls.

FIGURE 5



The data generated by the virus yield assays are not interpretable regarding the efficacy of AG17 as an anti-viral agent (*See* upper graph in Figure 5). While AG17 may possess antiviral activity, as indicated by reduced plaque formation, the observed reduction in virus yield

mediated by this agent at all tested concentrations may simply be due to its cellular toxicity, i.e. HSV cannot replicate in cells that have been killed by AG17. I am aware of no subsequent publications, by Yura *et al.* or otherwise, making the correlation described by Yura *et al.*, regarding the use of tyrophostins as anti-viral therapeutics.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

26 June, 2003
Date

W. James Waldman
W. James Waldman, Ph.D.

Attorney Docket No. 29666/35415A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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June 24, 2003

Michael F. Borun, Reg. No. 25,447
Attorney for Applicants

Declaration of Edward S. Mocarski, Jr., Ph.D.
under 37 C.F.R. 1.132

Commissioner of Patents and Trademarks
Patent and Trademark Office
Washington, D.C. 20231

Dear Sir:

I, Edward S. Mocarski, Jr., Ph.D., declare that:

1. I am a professor in the Department of Microbiology and Immunology at Stanford University School of Medicine where I have been employed since 1983. During my tenure at Stanford, I have been the Chair of the Department of Microbiology and Immunology (1994-1999) and the Associate Dean of Research (2000-2001). I have had two six-month sabbaticals, taking one at Systemix and one at Aviron. Prior to joining Stanford, I completed my post-doctoral training in virology at the University of Chicago, where for three years thereafter, I was a Leukemia Society of American Special Fellow. I received my Ph.D. in Microbiology from the University of Iowa after earning my A.B. in Microbiology from Rutgers University. I have held at least 16 panel memberships, been a US District Court-appointed expert and am currently on the editorial boards of the Journal of Virology, Virology and the Journal of Biological Chemistry. Presently, my laboratory is funded by six NIH grants to investigate various immune responses and the causes and treatments thereof focused on cytomegalovirus, including, DNA

replication, inversion, pathogenesis, latency and gene regulation. I have more than 100 publications and remain extremely active in the professional meetings and committee organizations with an emphasis on cytomegalovirus and herpesvirus.

2. I have read the article entitled, "An Evaluation of Leflunomide in the Canine Renal Transplantation Model" by McChesney, *et al.*, published in June of 1994 in Transplantation, Vol. 57, No. 12, 1717-1722 ("McChesney") and am qualified to discuss its teachings regarding viral infection on the basis of my experience and training.

3. McChesney evaluated the immunosuppressive abilities of three drugs in the prevention of kidney allograft rejection in a canine model: leflunomide (a synthetically produced isoxazole derivative that bears no structural resemblance to existing immunosuppressive drugs), the active metabolite of leflunomide (A77-1726), cyclosporine (a common immunosuppressant that inhibits lymphocyte activation), as well as the synergistic combinations thereof.

4. According to the results in McChesney, leflunomide provided an increasing immunosuppressive effect in the canines correlative with dosage increases (although the highest dosage of 16mg/kg/day was toxic, a dose demonstrated to be tolerable in humans). When leflunomide was co-administered with cyclosporine to six dogs, all exhibited significant improvement in survival as compared with the survival data from the canines in each of the other groups. The immunosuppressive effect of leflunomide in this co-administration was evaluated by ceasing leflunomide administration in two animals after post-operative day 119, without discontinuing the cyclosporine. Within 14 days both canines developed fatal allograft rejection. The active metabolite of leflunomide (A77 1726) was also tested in eight canines. No significant increase in immunosuppressive effect was observed (as determined by a comparison between the overall survival rate of this group to the canine control group).

5. McChesney does not describe a study designed to evaluate antiviral effects. The publication contains no description of any procedure for virally challenging the canines and assessing the corresponding antiviral effects. Such a design is essential in an investigation of antiviral properties of a drug. There are also no virologic data presented in the paper, i.e. no

evidence that the investigators quantitatively monitored viral load through the study period. The only mention in McChesney to viral infection is a statement in the abstract that, "Even at a high dose of 16mg/kg/day no viral or bacterial infections were noted." There are no data reported in the article to support or explain this statement, which would be necessary in order to attribute such an observation to the administered drugs. Further, with respect to this single statement in the abstract, it addresses only leflunomide, as neither the metabolite nor cyclosporine were administered at a dosage of 16mg/kg/day. I am not however surprised that McChesney noted this lack of viral infection. Institutional animal care guidelines (followed by McChesney according to page 1721) require full vaccination of animals. I would have no reason to expect that these animals were not fully vaccinated since there is nothing in the study protocol suggesting a plan to evaluate the antiviral activities of the study drugs.

6. I was asked to consider whether the statement on page 1720, that one canine developed symptomatic kennel cough, suggested antiviral effects of leflunomide, i.e. were other canines protected from infection by leflunomide, its metabolite, or cyclosporine? There is insufficient information to suggest this conclusion. For example, was this canine an untreated control or did it receive leflunomide, the metabolite, cyclosporine or a combination thereof? Since the main cause of kennel cough is the airborne bacterial organism *Bordetella bronchiseptica*, this observation bears absolutely no relevance to the issue of antiviral activity. For these reasons, the reference to kennel cough does not suggest that McChesney reports that any of the study drugs act as antiviral agents. Importantly, McChesney makes no claim or even a suggestion that any of the drugs used in this study may possess antiviral properties, nor do the investigators suggest the use of the study drugs as antiviral agents.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Edward J. Mocarski Jr. — April 2, 2003

Date — Name